KING EIDER WING MOLT: INFERENCES FROM STABLE ISOTOPE ANALYSES

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A

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Abstract: The western North American population of the King Eider is thought to have declined by over 50% between 1974 and 1996 without an apparent cause. The non-breeding period of King Eiders consists of 80-100% of their annual cycle if not impossible by observation. I used stable carbon and nitrogen isotope values of feathers and muscle to examine the wing molt and migration ecology of King Eiders in 2003. Eider primary feathers were isotopically homogenous along the length of the feather, implying invariable diets during wing molt. Captive eiders in their hatch-year did not fractionate nitrogen isotopes, potentially indicating preferential protein allocation associated with growth. Six percent of female eiders sampled molted primary feathers on their breeding grounds, which had not been previously substantiated. Tissue samples from both genders corroborated dietary shifts inherent in switching from a marine to terrestrial diet. Carbon isotopes of feathers from satellite-transmittered males were correlated with longitude of their known wing molt locations indicating that the gradient of carbon isotopes can be used to draw inferences about molt location of eiders.

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INTRODUCTION

Stable isotope analyses are commonly used to examine trophic ecology (Kelly 2000) and migration (Hobson 1999, Rubenstein and Hobson 2004) in avian ecology. Atmospheric sources of carbon (Craig 1953) and nitrogen (Junk and Svec 1958) exist in nearly invariable ratios which are altered through chemical processes in trophic systems (McConnaughey and McRoy 1979, Wada 1980, Tieszen et al. 1983, Minagawa and Wada 1984, Petersen and Fry 1987). Locations of migratory stopovers, wintering areas, and other aspects of a species' annual cycle are poorly understood; however, knowledge of these locations is essential for long term management of wildlife populations (Webster et al. 2002). Stable carbon isotopes have been used successfully to track (Marra et al. 1998) and identify geographic origins of migratory routes (Wassenar and Hobson 2001). King Eiders (*Somateria spectabilis*) are a species of concern in the U.S.A. due to a nearly 50% multi-decade population decline (Mosbech and Boertmann 1999, Suydam et al. 2000). King Eiders are difficult to study because they are at sea for 80 -100% of their annual cycle (Bellrose 1976); thus, applications of stable isotope analyses to gather baseline information on non-breeding ecology of King Eiders are useful.

Stable isotopes of an element exist in nature in several different forms (Petersen and Fry 1987) and can be used as tools to examine trophic ecology of animals because isotopic values of tissues from animals reflect those of their diet (Hobson and Welch 1992, France and Peters 1997). Isotopically heavier forms of C and N (^{13}C and ^{15}N) are less abundant than the lighter forms (^{12}C and ^{14}N) (Craig 1953, Junk and Svec 1958). Through the process of fractionation, heavier isotopes are differentially discriminated

against while being processed by the consumer. Fractionation of isotopes in substrates can be best understood in terms of chemical rates of reaction (Petersen and Fry 1987). Chemical substrate pools from the diet contain heavy and light isotopes. Lighter isotopes of particular elements have higher free energy and are used more quickly, but, as the lighter isotope becomes scarcer, the heavier isotopes are increasingly incorporated, thus generating a similar isotopic ratio between consumer tissues and their diet. However, previous studies demonstrate that values of tissues from animals fed a uniform diet are isotopically heavy compared to, or "enriched" above, their diet. Typical enrichment factors are 1‰ for $\delta^{13}C$ (Deniro and Epstein 1978, McConnaughey and McRoy 1979, France and Peters 1997, France et al. 1998) and 3-5‰ for $\delta^{15}N$ (Deniro and Epstein 1981, Minagawa and Wada 1984, Evans Ogden et al. 2004). Enrichment in carbon may be due to preferential respiration of ^{12}C, preferential uptake of ^{13}C during digestion, or preferential incorporation of ^{13}C during tissue synthesis (Deniro and Epstein 1978, Rau et al. 1983, Tieszen et al. 1983). Nitrogen enrichment is generally thought to result from preferential excretion of ^{14}N (Minagawa and Wada 1984).

Differences in the stable isotopic composition of animal tissues are generally the result of differences in the isotopic composition of diets. Different locations often differ in the dominant biological processes (for example, photosynthetic pathway, trophic interactions) (Deniro and Epstein 1981, Fry and Scherr 1984, Minagawa and Wada 1984, Hobson 1995). Terrestrial carbon sources vary depending on the photosynthetic pathway used by primary producers. C_3 plants have depleted $\delta^{13}C$ signatures because they preferentially discriminate against uptake of ^{13}C, and C_4 plants are enriched in ^{13}C

relative to C_3 plants because of their highly efficient CO_2 uptake (Fry and Scherr 1984). Marine carbon sources are enriched in ^{13}C relative to terrestrial plants, because presumably there is little to no isotopic discrimination against ^{13}C during uptake of available CO_2 in marine plants (Andrews and Abel 1979). Generally, marine nitrogenous compounds are ~7‰ enriched above atmospheric nitrogen (Miyake and Wada 1967). As primary producers are consumed by herbivores, there is a step-wise enrichment in $\delta^{15}N$ of tissues that continues at a fairly constant rate at each increase in trophic level (Minagawa and Wada 1984, Hobson et al. 1994, France and Peters 1997). However, enrichment factors vary among organisms in similar trophic levels because predators and omnivorous species consume a variety of prey that occupies different trophic levels (Hobson and Welch 1992, Bearhop et al. 1999). These factors allow investigators to differentiate among marine, terrestrial, or mixed sources (Mizutani et al. 1990, Naidu et al. 1993, Bearhop et al.1999, Hobson 1999) and the trophic level for an organism or community (Hobson 1993, Hobson et al. 1994, Schmutz and Hobson 1998).

Differences in the stable isotope composition of tissues within an individual may vary over time with diet. These differences result from variable rates of tissue-specific protein turnover as a consequence of diet change (Tieszen et al. 1983, Hobson and Clark 1992a, 1992b, Hobson 1993, Haramis et al. 2001). For instance, turnover rates in liver are rapid relative to muscle; therefore values of liver may represent the recent diet while muscle may represent diet integrated over weeks or months. However, keratin-based tissues, such as feathers, do not turnover protein after synthesis (Mizutani et al. 1992, Hobson 1999, Pearson et al. 2003). Thus, metabolically active tissues reflect a current or

recent diet and metabolically inactive tissues depict the dietary isotopic composition at the time of synthesis.

Previous studies of marine invertebrates have demonstrated that stable isotopes vary geographically in nature (Schell et al. 1998) and that geographic gradients exist for $\delta^{13}C$ in the arctic seas used by King Eiders (Dunton et al. 1989, Saupe et al. 1989). The diet of King Eiders at sea consists mainly of marine invertebrates (Lamonthe 1973, Frimer 1997). Given that the isotopic composition is known for some marine invertebrates in the Bering and Beaufort Seas, inferences regarding the trophic ecology and migration may be drawn from stable isotope values of tissues from eiders based on known patterns of stable isotope distributions in their likely prey.

The nature of isotopic fractionation and distributions of stable carbon and nitrogen isotopes within and among ecosystems allows for their use in studying migration (Hobson 1999, Kelly 2002, Rubenstein and Hobson 2004) and non-breeding biology (Alisauskas and Hobson 1993, Cherel et al. 2000). Traditional methods in avian studies such as mark-recapture do not necessarily yield adequate information about migratory routes or wintering biology of a particular species because recapture/resighting probabilities are low. Weight restraints and expense limit sample sizes in satellite telemetry studies (Webster et al. 2002); therefore, transmittered individuals may not represent the entire population.

In this study I examined the stable isotopic composition of primary feathers and muscle tissue from King Eiders to better understand their wing molting ecology and migration. Given the limited information regarding the annual cycle of King Eiders, their

population decline, and the recent interest in management of sea ducks (Sea Duck Joint Venture Board 2001), I constructed the following objectives:

1. Determine cleaning method with the least effects on the feather $\delta^{13}C$ and $\delta^{15}N$ values,

2. Determine the variability of isotopic composition along the feather length,

3. Examine effects of gender or age on isotopic fractionation from diet to feathers in captive eiders reared on a uniform diet,

4. Determine whether $\delta^{13}C$ and $\delta^{15}N$ values of feathers from King Eiders could be explained by diet at wing molt location and/or gender,

5. Compare $\delta^{13}C$ and $\delta^{15}N$ values in muscle and feather tissues that might reflect shifts in diet from a marine-based diet during the non-breeding period in winter to a terrestrial based diet during breeding in summer,

6. Determine whether $\delta^{13}C$ values in feathers from transmittered eiders are correlated with longitude of known molt locations in the Bering Sea.

I formulated these objectives for several reasons. First, a survey of the stable isotope literature using feathers revealed that five methods have been used to remove feather surface contaminants (e.g. Mizutani et al. 1992, Chamberlain et al 1997, Wassenaar and Hobson 2000, Pearson et al. 2003, and Pain et al. 2004). Surface contaminants could have different isotopic composition than feathers and failing to remove them may bias the stable isotope values of the analyses (Hobson and Clark 1992a). No studies using feathers have validated methods used to clean them, and,

therefore, there is no consensus for cleaning methods. Second, I hypothesized that $\delta^{13}C$ and $\delta^{15}N$ values of feathers would represent the diet of captive King Eiders during the 3-4 week molting period. Consistent stable isotopic composition of feathers along the entire feather length from captive and wild eiders would indicate that their diets during feather synthesis were also consistent. Geographic restriction during wing molt is an important prerequisite to make inferences about the distribution of King Eiders during this period. Should $\delta^{13}C$ and $\delta^{15}N$ values of feathers from wild eiders vary along the length, it would indicate that eiders diets were changing during wing molt and that potentially they were not geographically restricted during the period of feather synthesis. Third, I reasoned that if there were differential fractionation rates from diet to feathers in captive eiders of known age and gender, these differences would need to be considered in the interpretation of stable isotope values of feathers from wild eiders. Fourth, I hypothesized that King Eiders may molt in different geographic locations separated by gender similar to Steller's eiders (*Polysticta stelleri*) (Flint et al. 2000) or there may be biological factors such as differences in metabolism by gender associated with reproduction (Nagy 1987) that might alter stable isotope values of feathers. Fifth, I hypothesized that muscle tissue in eiders that migrated from the Bering Sea and staged in the isotopically depleted Beaufort Sea or attempted breeding in terrestrially depleted areas would have lower $\delta^{13}C$ and $\delta^{15}N$ values of muscle in the fall than the spring. Finally, I hypothesized that due to consistent patterns of stable isotope distributions and trophic enrichment, that the $\delta^{13}C$ values of feathers from satellite transmittered eiders would be correlated with the longitude of their known wing molt location.

This thesis consists of two sections. The first chapter examines the effects of cleaning methods on stable carbon and nitrogen isotope values of feathers, stable isotopic composition of primary feathers, and gender and age effects on isotopic fractionation in captive King Eiders (Objectives 1-3). The second chapter examines variation in the stable carbon and nitrogen isotope values of feathers due to gender or location of molt, changes in the diets of King Eiders after wintering and migrating to breeding areas, and the correspondence of stable carbon isotope values of feathers from King Eiders implanted with satellite transmitters and the longitude of their known wing molt location (Objectives 4-6). Finally, in the third section, I will draw conclusions regarding both chapters and make recommendations for future studies.

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CHAPTER 1. VARIATION IN THE $\delta^{13}C$ AND $\delta^{15}N$ COMPOSITION OF FEATHERS FROM KING EIDERS: EFFECTS OF CLEANING AND BIRD AGE[1]

Abstract. We examined the effect of cleaning methods on $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers and variation in isotopic fractionation due to age and sex in King Eiders (*Somateria spectabilis*). First, we found that feathers cleaned with ethanol, soap and water, and soap and chloroform/methanol solutions did not differ in $\delta^{13}C$ and $\delta^{15}N$ values by method. We therefore recommend ethanol as a reliable method of cleaning feathers for isotopic measures. Second, we compared $\delta^{13}C$ and $\delta^{15}N_{\text{diet-feather}}$ values among ages and between genders. We found no gender-related differences in stable isotope values of feathers. Feathers of adult eiders ($\delta^{13}C$ -18.63 ± 0.40‰; $\delta^{15}N$ 12.07 ± 0.69‰) were significantly enriched above their diet ($\delta^{13}C$ -21.44 ± 0.57‰; $\delta^{15}N$ 7.77 ± 0.42‰). Mean $\delta^{13}C$ values of feathers from hatch-year eiders (-19.10 ± 0.21‰) were higher than their diet (-21.51 ± 0.38‰) but feathers and diet were similar for $\delta^{15}N$ values (feathers 5.86 ± 0.46, diet 6.41 ± 0.28‰). Feather $\delta^{15}N$ values from hatch-year eiders were lighter than those from adults even though dietary C, N and S contents were similar. Small differences between hatch-year and adult birds in sulfur content of feathers probably

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reflect differences in keratin composition during growth. To our knowledge, this is the first study to demonstrate age-dependent fractionation from diet to feather in birds. Discrimination of hatch year and adult feathers in samples from wild birds may increase the ability to identify diets in each age group.

Key words: *diet, feathers, fractionation, keratin,* Somateria spectabilis, *stable isotope*

INTRODUCTION

Trophic ecology and various aspects of non-breeding biology of sea ducks and seabirds are difficult to monitor because these species spend a large proportion of their annual cycles in locations that are either unknown or logistically difficult to access (Goudie and Ankney 1988, Guillemette et al. 1993, Hobson 1993). The non-breeding biology (i.e., staging, molting, wintering) of King Eiders is of growing interest because their populations may have declined by more than 50% since the early 1970's for unknown reasons (Suydam et al. 2000, Suydam 2000).

Stable isotope analyses provide natural markers to examine trophic ecology and movement of animals throughout the annual cycle (Webster et al. 2002, Rubenstein and Hobson 2004) and are widely used across taxa with particular utility for avian systems (reviewed in Kelly 2000). Interpretation of stable $\delta^{13}C$ and $\delta^{15}N$ isotope values must consider general sources of variation such as individual, sex, and age as well as variation specific to tissue type. The $\delta^{13}C$ and $\delta^{15}N$ values of animal tissues are affected by turnover rates of elements within a tissue and by the source of elements. Sources of

carbon (C) and nitrogen (N) can vary with diet (Hobson and Clark 1992a, Hobson and Welch 1992, Hobson 1993, Hobson et al. 1994) or location (Hobson 1990, Bearhop et al. 1999). Tissue turnover varies among individuals or between sexes because of variable energy requirements and net synthesis of tissues owing to maintenance, growth, or reproduction (Nagy 1987). Several applications of $\delta^{13}C$ and $\delta^{15}N$ analyses to avian biology have concluded with a call for more laboratory studies to better understand biological processes affecting stable isotopic fractionation (Hobson and Clark 1992b, Gannes et al. 1997, Bearhop et al. 1999, 2002, Webster et al. 2002).

Age-dependent fractionation has been suspected but not directly examined experimentally. While some studies contend that there is no age-dependent fractionation in birds (Mizutani et al. 1992), others have noted variability in stable isotope composition among different age groups and have suggested effects due to age (Hobson and Clark 1992b, Graves et al. 2002).

Different rates of elemental turnover within and among tissues can be used to time trophic or geographic shifts in the source of C and N (Schmutz and Hobson 1998, Evans Ogden et al. 2004). Feathers are a good indicator of C and N sources because feather keratin is composed of collagens which exhibit minimal variance within tissue (Stettenheim 1972, Murphy 1996a). Temporal patterns of molt are predictable for easily discernible types of feathers and synthesized keratins are not re-utilized, thus indicating the source of C and N at the time of feather synthesis (Hobson and Clark 1992a, Mizutani et al. 1992, Cherel et al. 2000, Pearson et al. 2003).

Surface contaminants may alter apparent feather $\delta^{13}C$ and $\delta^{15}N$ values. The removal of contaminants such as exogenous oils and dirt as well as secretions such as uropygial oils may be a prerequisite for clearly identifying dietary sources of C and N during the formation of feathers. Researchers have removed surface contaminants using water (Mizutani et al. 1992), soap and water (Chamberlain et al 1997, Meehan et al. 2003), sodium hydroxide (Bearhop et al. 2002, Pain et al. 2004), ether (Hobson and Clark 1992a, Pearson et al. 2003), and mixtures of chloroform and methanol (Wassenaar and Hobson 2000, Cherel et al. 2000, Hobson et al. 2003, Smith et al. 2003, Mehl et al. 2004). A comparison among cleaning methods would facilitate some consensus on a standard cleaning method for removing surface contaminants from feathers prior to isotopic measurements.

Several aspects of eider biology establish this group of sea ducks as a strong candidate for stable isotope analyses. First, King Eiders experience synchronous primary feather molt (Bellrose 1976) which prevents flight and restricts the diet to sources within a small geographic range where they consume marine invertebrates that are rich in protein (Frimer 1997). The high availability of amino acids in diets of King Eiders facilitates rapid incorporation of C and N in feathers during molt. Consequently, $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers should represent the diet and geographic area where molt occurred.

In this study, we first used wild and captive King Eiders to examine effects of cleaning on the feather $\delta^{13}C$ and $\delta^{15}N$ values and to examine the degree of uniformity of

feather composition. We then examined effects of sex or age on isotopic fractionation from diet to feathers in captive eiders reared on a uniform diet.

METHODS

ANIMAL HUSBANDRY

Captive King Eiders were reared at Dry Creek Waterfowl, Port Townsend, Washington. Young eiders in their hatch year (HY) were fed Mazuri Waterfowl Starter (PMI Nutrition International, St. Louis, MO) and held in a small covered pen for the first five weeks post-hatch until primary feathers were completely formed. Adult birds after hatch year (AHY) were maintained along with several other species of sea ducks in three large outdoor ponds of approximately 800 m^2 under cover of nylon netting. Both HY and AHY eiders were fed Mazuri Sea Duck diet pellets *ad libitum* after five weeks of age.

CLEANING METHODS

We collected the ninth primary feather from all birds to eliminate any variation among different primary feathers. Feathers were collected from one captive AHY male, one AHY female, and one HY female after wing molt had occurred in November 2002. Additionally, we sampled primaries from one AHY male and two AHY females that were shot by subsistence hunters at Pt. Barrow, AK (71° 20.5′ N, 156° 44′ W) in May and August 2003. Primary feathers collected at Pt. Barrow were presumably molted in 2002 based on the annual molt cycle of King Eiders (Frimer 1994).

We used a repeated measures approach to test two cleaning methods on opposite sides of the same feather. Primary feathers from the six eiders were cut lengthwise into two halves with a scalpel blade. Feather barbs were sampled at four points along the length of the feather vein in increments of 2 cm from the base to the tip of the feather. One side of the feather was washed with Dawn© (Proctor and Gamble) dish soap and water, scrubbed with a cotton swab, and then rinsed with water. The other side was washed with ethanol, scrubbed with a cotton swab, and then rinsed again with ethanol. All samples were washed until no residual material could be seen on the cotton swab and allowed to air dry before weighing.

Feathers previously cleaned with soap and water were also cleaned and rinsed with a 2:1 solution of chloroform: methanol to remove any additional oils or residues left behind by the soap. We compared the $\delta^{13}C$ and $\delta^{15}N$ values of feathers treated with soap and water with the subsequently washed samples to assess if the prior treatment with soap had left any residues. We compared ethanol-washed feathers and chloroform/methanol treated feathers to determine if there was any effect of the latter residues on feather $\delta^{13}C$ and $\delta^{15}N$ values.

We measured ethanol soluble components of unclean feathers by placing approximately 0.5 g of primary feather (~4 primaries) into polyester mesh bags (ANKOM Technology). Sample bags were thoroughly washed by frequent agitation in three sequential volumes of ethanol over 24 h. Sample bags were dried at room temperature then oven dried at 90°C for 48 hours to determine final dry mass. Ethanol

soluble material was estimated as the loss in dry mass in each sample after correcting for change in mass of empty sample bags.

EFFECTS OF AGE AND SEX

We used ethanol to clean feathers for the remainder of the study because it is inexpensive and easier to use and transport than chloroform. We collected feathers from eleven female HY and eight (four male and four female) AHY captive eiders aged 2, 3, 4, 6, 7 (2), and 11 (2) years. These feathers were collected post-wing-molt in November and December 2003 to examine sex and/or age-associated influences over the $\delta^{13}C$ and $\delta^{15}N$ composition of feathers.

Diet samples for adult birds were collected from Dry Creek Waterfowl in August, September, and October 2003 to examine isotopic variation that may occur because of potential ingredient swapping in commercially produced foods (Haramis et al. 2001). We ran three samples from each batch for stable carbon and nitrogen isotopes. Pellets from the waterfowl starter for HY birds were only available in August 2003. Diet pellets were ground into a fine, homogenous powder with mortar and pestle.

STABLE ISOTOPE ANALYSES

Stable isotopes were measured by continuous flow stable isotope-ratio mass spectrometry (CFSI-RMS). Feather samples from both experiments were analyzed using a Carlo Erba NC2500 elemental analyzer (Carlo Erba, Milan, Italy) attached to a Finnigan Delta+XP (Thermo Electron Corporation, Waltham, MA). Stable isotope ratios were reported in δ

notation, as parts per thousand (‰) relative to Pee Dee Belemnite (PDB) (carbon) and atmospheric air (nitrogen), using the following equation:

$$\delta X = ([R_{sample}/R_{standard}]\text{-}1 \text{ X } 1000,$$

where X is ^{15}N or ^{13}C and R is the ratio of ^{15}N:^{14}N or ^{13}C:^{12}C. Instrument precision was determined by conducting multiple analyses of a homogenous calibrated peptone standard ($n = 29$) ($\delta^{13}C = 7.0‰$, C = 44.3%, $\delta^{15}N = 15.8‰$, N =15.8%) and were $< \pm 0.4$ ‰ for $\delta^{13}C$, $< \pm 0.3$ ‰ for $\delta^{15}N$.

PROXIMATE ANALYSES

Feathers and diet samples remaining after they had been subsampled were dried to a constant mass in a fan-forced oven and ground with a Wiley mill for analysis (Barboza and Jorde 2001). We used procedures for proximate analysis described by Barboza and Jorde (2002) to determine ash by combustion, and determined C, N, and sulfur (S) content with a LECO (LECO Corporation, St. Louis, MO) elemental analyzer.

CALCULATIONS AND STATISTICAL ANALYSES

Cleaning methods were compared as relative to ethanol as our standard. Within feather normalization of $\delta^{13}C$ and $\delta^{15}N$ values removed the large variation between young and adult birds in the cleaning experiment. We compared cleaning treatments at sites along the length of the feather among individual feathers (i.e., five locations on six feathers)

and within each individual feather (i.e., the five locations within each feather) using repeated measures ANOVA (SYSTAT Version 10.2; SYSTAT Software, Richmond CA). Our model included variables for Site (cm distance from base of feather), Status (wild vs. captive), and Treatment (cleaning method). Pairwise comparisons between sites were performed with Bonferroni's adjustments. We used ANOVA to examine fractionation differences between sexes and among ages of AHY eiders, HY and AHY feather stable isotope composition, and proximate analysis of diet and feather. Means are reported with ±1 S.D.

RESULTS

CLEANING EXPERIMENT

Cleaning treatments did not affect $\delta^{13}C$ and $\delta^{15}N$ values in feathers (Fig. 1). Thorough washing with ethanol only dissolved 4.73 ± 0.28 mg dry mass per 100 g of feather. Sampling locations on individual feathers did not differ significantly in $\delta^{13}C$ and $\delta^{15}N$ values, that is, feathers were isotopically homogenous for C and N (Table 1).

DIET AND FEATHER

The diets of AHY and HY eiders were similar in nutrient composition (Table 2) with only an 8% reduction in dietary N in the HY diet compared with that of the adults ($n = 8$, $P < 0.05$, Table 2). Diet $\delta^{13}C$ and $\delta^{15}N$ values were consistent between sub-samples and indicated a constant stable isotopic source for each age group. Diets were similar in $\delta^{13}C$

values but the HY diet was 18% lighter in $\delta^{15}N$ than the AHY diet ($n = 12$, $P < 0.01$, Fig. 2).

Feathers from AHY eiders were significantly higher in $\delta^{13}C$ (2.82 ± 0.40‰) and $\delta^{15}N$ (4.30 ± 0.69‰) than their diet ($n = 19$, $P < 0.001$ for $\delta^{13}C$ and $\delta^{15}N$, Fig. 2). HY feather $\delta^{15}N$ values (-0.55 ± 0.46‰) were similar to the diet (Fig. 2) even though $\delta^{13}C$ values were significantly higher (2.41 ± 0.21‰) in feathers than diet ($n = 12$, $P < 0.001$).

Feather $\delta^{13}C$ and $\delta^{15}N$ values did not vary with age or sex among AHY birds between 2 - 11 years of age. The HY feather $\delta^{15}N$ values were significantly lower than feathers from all AHY eiders ($n = 19$, $P < 0.001$, Fig. 2) even though $\delta^{13}C$ values of feathers were similar between HY and AHY birds. Stable isotopic differences in feathers between age groups were not due to differences in C and N content (Table 2). Feathers from HY birds were, however, lower in S than feathers from adults ($n = 19$, $P < 0.005$, Table 2).

DISCUSSION

Early studies of stable carbon isotopes demonstrated that lipids tend to be depleted in ^{13}C relative to other tissues (Deniro and Epstein 1977) and it was suggested that removing lipids from feathers may improve analytical precision for feather stable isotope signatures produced at the time of feather synthesis (Hobson and Clark 1992a). However, Mizutani et al. (1992) found that the presence of uropygial oil did not alter $\delta^{13}C$ values of feathers and determined that cleaning feathers with water was not necessary. Cleaning with water may not remove hydrophobic materials such as oils. In our study, thorough cleaning with

chloroform/methanol removed most lipids but still did not alter feather $\delta^{13}C$ and $\delta^{15}N$ values. The consistency of average feather stable isotope values among treatments probably reflects the small amount of surface contamination in these samples that was less than 0.5% of dry mass when removed with ethanol.

Feather cleaning may be an important prerequisite for stable isotope analysis especially when samples are collected from wild birds, because extraneous oils or solids can adhere to the sample during excision or transport in the field. Contamination with blood or secretions from the bird may also require cleaning prior to analysis, especially when birds are shot or collected by hunters. Wild King Eiders migrate between two the Bering Sea and Beaufort Sea, which are known to be isotopically distinct (Schell et al. 1998, Hoekstra et al. 2002). Surface contamination of feathers with blood or uropygial oils synthesized from diets at one site may therefore alter $\delta^{13}C$ and $\delta^{15}N$ values of uncleaned feathers synthesized at the other.

Homogeneity of the stable isotopic composition along the length of the feather generated two implications for stable isotope studies using feathers. First, C and N inputs from endogenous and exogenous sources during feather synthesis appear to be consistent Table 1). Though molt is energetically stressful in birds (Payne 1972, King 1980) and protein demands are high (Murphy 1996b), feather growth proceeds at a fairly constant rate (Robbins 2001). Feather structures such as barbs are probably similar in composition of keratins throughout the synthesis of the feather and, thus, we might expect consistent $\delta^{13}C$ and $\delta^{15}N$ values of barbs along the shaft. Second, homogenous $\delta^{13}C$ and $\delta^{15}N$ values

along the feather implies that dietary sources of C and N do not change during wing molt in wild eiders.

The diets of AHY and HY eiders during the period of primary feather growth were very similar in percentages of dry matter and stable isotope composition. Small differences in dietary N should not have affected $\delta^{15}N$ values or composition of feathers in eiders because both diets were adequate for growth and reproduction of domestic poultry (NRC 1994). Dietary content of N may not affect ^{15}N composition of tissues unless the total intake of N limits net synthesis of proteins (Pearson et al. 2003). The $\delta^{15}N$ values of feathers in water birds such as gulls, cormorants, ibis and flamingos were similarly related to dietary $\delta^{15}N$ values and independent of dietary N when those intakes were apparently adequate for growth and synthesis of feathers (Mizutani et al. 1992). Feather $\delta^{13}C$ and $\delta^{15}N$ values could change, however, when molting birds switch between adequate and inadequate intakes of N, for example when diets switch from carnivorous (high N) to frugivory (Pearson et al. 2003). Small differences between HY and AHY eiders in S content of feathers probably indicate ontogenetic changes in keratin composition (Table 2). The lower sulfur content of HY feathers probably reflects differences in protein sequences of keratins that vary among types of feathers, scales, and claws within and among species of birds (Busch and Brush 1979; Stevens 1996).

We suggest that the lack of $\delta^{15}N_{\text{diet-feather}}$ enrichment in HY eiders results from retention of N from the diet. Other studies of captive birds have determined $\delta^{15}N$ to increase in tissues above the diet because ^{14}N is preferentially excreted (Pearson at el. 2003, Vanderklift and Ponsard 2003). Rapid growth in HY eiders may increase N

demands such that resulting net N loss is minimized and there is no enrichment from diet to tissue in HY eiders. The $\delta^{15}N_{diet\text{-}feather}$ values from adult eiders (4.30 ± 0.69‰) were within the range found by Mizutani et al. (1992) (3.7 - 5.3‰) and Pearson et al. (2003) (3.2 - 4.3‰), thus corroborating previous patterns of $\delta^{15}N_{diet\text{-}feather}$ fractionation found in other species. The similarity of $\delta^{13}C_{diet\text{-}feather}$ values between HY and AHY King Eiders probably reflects similar C sources in their respective diets.

Some researchers have observed differences in the values of stable isotopes between the tissues of wild HY birds and their parents. Those age-dependent differences have been attributed to differences in diet between parents and their offspring (Schmutz and Hobson 1998, Hodum and Hobson 2000, Graves et al. 2002) or to differences in the location of feather molt and, thus, feeding site (Cherel et al. 2000). Further comparisons of $\delta^{13}C$ and $\delta^{15}N$ values between growing and adult birds within studies or between data sets should consider concomitant changes in the deposition and flux of both C and N that may alter the fractionation of each isotope between diet and feathers.

Finally, $\delta^{13}C_{diet\text{-}feather}$ values from HY (2.41 ± 0.21‰) and AHY (2.82 ± 0.40‰) eiders were above typical $\delta^{13}C$ enrichment values (≤ 1‰) from metabolically active tissues (Tieszen et al. 1983, McCutchan et al. 2003). We speculate that the isotopic composition of individual amino acids may account for greater enrichments in feathers than other tissues. In domestic pigs, the $\delta^{13}C$ values of individual amino acids in their diets ranged from -27.4 to -13.8‰, and their corresponding values in tissues reflected those differences (Hare et al. 1991). Feathers are composed primarily of the sulfur-containing amino acids methionine and cysteine (Murphy 1996a). Though, to our

knowledge, there are no published $\delta^{13}C$ values for methionine and cysteine, we propose that higher enrichments in sulfur-containing amino acids could explain higher enrichments in feathers.

CONCLUSIONS

We conclude that: (1) removal of surface contaminants from feathers prior to isotopic analyses can be done easily using ethanol, (2) the stable C and N isotopic composition of primary feathers represent the diets of AHY King Eiders during wing molt, and (3) diet-feather fractionation may differ between HY and AHY birds in the wild. Separation of HY and AHY birds in sample sets from wild birds may increase the ability to identify sources of dietary N or trophic position during the annual cycle of King Eiders.

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Table 1. Homogenous isotopic composition of primary feathers from eiders. Mean $\delta^{13}C$ and $\delta^{15}N$ values of feathers from King Eiders ($n = 6$) cleaned with ethanol at five locations along the length of the feather. Eiders are classified by after hatch-year (AHY) or hatch-year (HY), male (M) or female (F), and wild (W) or captive (C).

Individual	Source	$\delta^{13}C$	$\delta^{15}N$
AHY-M	W	-16.10 ± 0.21	13.92 ± 0.41
AHY-F	W	-18.54 ± 0.24	14.97 ± 0.57
AHY-F	W	-17.48 ± 0.23	14.07 ± 0.32
AHY-F	C	-18.33 ± 0.22	10.60 ± 0.30
AHY-M	C	-18.08 ± 0.15	10.74 ± 0.18
HY-F	C	-19.82 ± 0.50	5.60 ± 0.34

TABLE 2. Chemical composition of feathers from eiders and their diet. Values are presented as percent dry matter from captive hatch-year (HY) and after hatch-year (AHY) King Eiders.

	% C	% N	% S	Ash	Lipid
Diet					
AHY	44.13 ± 0.27	4.03 ± 0.03[a]	0.33 ± 0.01	12.06 ± 0.66	11.81 ± 0.33
HY	44.25 ± 0.09	3.95 ± 0.01[b]	0.31 ± 0.00	11.62 ± 0.36	11.00 ± 0.51
Feather					
AHY	49.37 ± 0.32	15.37 ± 0.14	2.73 ± 0.05[a]	0.31 ± 0.18	n/a
HY	48.31 ± 0.40	15.40 ± 0.18	2.66 ± 0.04[b]	0.27 ± 0.24	n/a

[a,b] Different superscript letters indicate significant differences between means ($P < 0.05$).

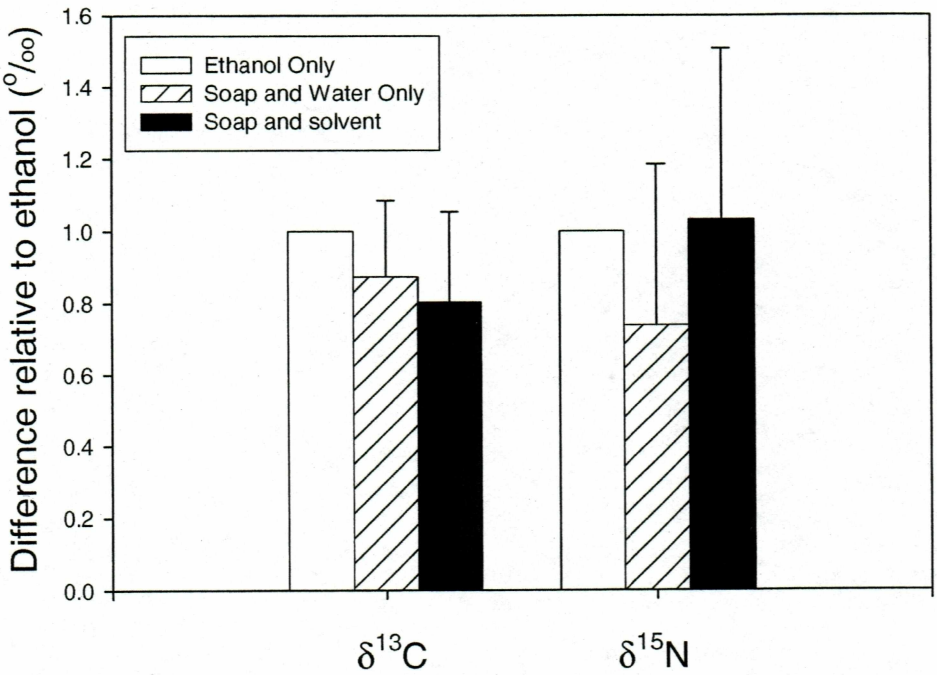

FIGURE 1. Effects of cleaning method on feather $\delta^{13}C$ and $\delta^{15}N$ values. Ethanol is normalized to one. The remaining bars represent the mean differences along the feather length between the values from ethanol cleaned sub-samples and soap and water or soap and chloroform cleaned feathers. The mean values represent five points from each of six feathers within each cleaning treatment. Error bars for comparison washes are the standard deviations for proportional change.

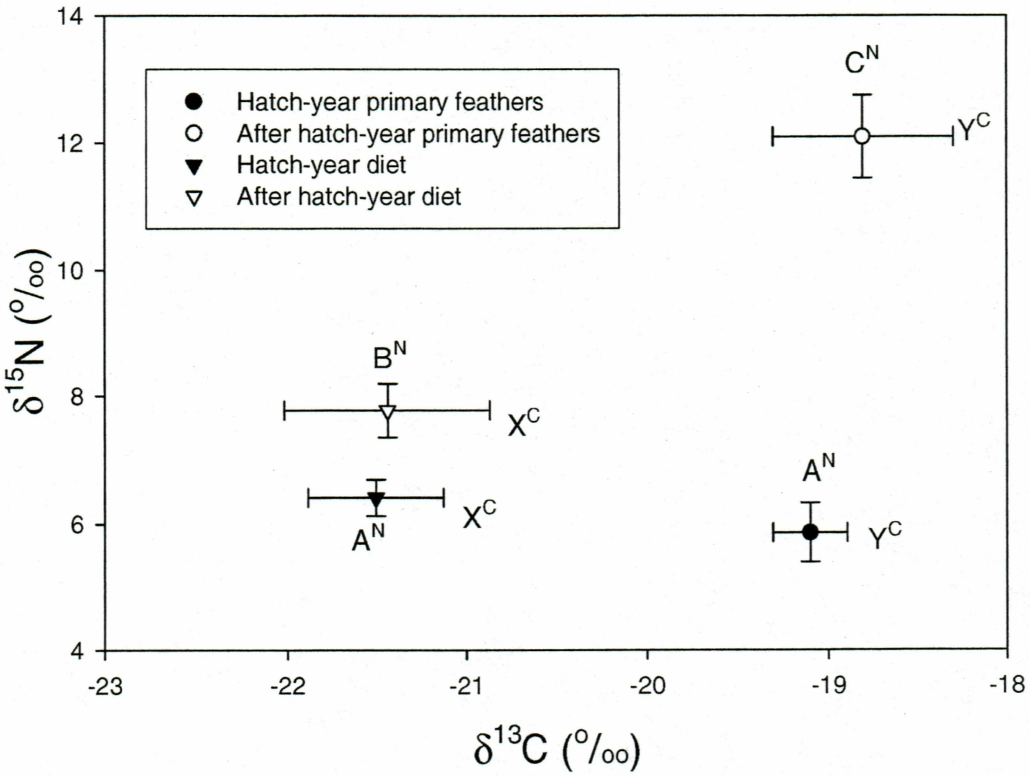

FIGURE 2. The $\delta^{13}C$ and $\delta^{15}N$ values of diet and feathers from captive eiders. Different letters with superscript C or N indicate significant differences between means for ^{15}N and ^{13}C, respectively. Mean ± SD; δ ‰

CHAPTER 2: KING EIDER WING MOLT AND MIGRATION: INFERENCES FROM STABLE CARBON AND NITROGEN ISOTOPES[1]

Abstract: We used the $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers from King Eiders (*Somateria spectabilis*) to examine variation in isotope signatures associated with location of molt or gender differences. We compared metabolically active (muscle) and inactive (feather) tissues to examine isotopic shifts inherent in switching from a marine-based diet (winter) to terrestrial-based diet (summer). Finally, we examined $\delta^{13}C$ and $\delta^{15}N$ values of feathers relative to known location of molt in King Eiders implanted with satellite transmitters. Six percent of female King Eiders had depleted $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers representing terrestrial molt. This is the first study to substantiate that a proportion of female King Eiders molted flight feathers in terrestrial areas. Feather $\delta^{13}C$ and $\delta^{15}N$ values did not differ among seasons. Feather and muscle $\delta^{13}C$ and $\delta^{15}N$ values did not differ in spring; however, muscle values were isotopically lighter in fall than spring and lighter than feather values in both seasons. $\delta^{13}C$ values of primary feathers from transmittered male King Eiders were positively correlated with

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their known longitude of molt. We also found evidence that dietary shifts occurring between wintering in a subarctic sea and breeding on the Arctic Coastal Plain, corroborate isotopic gradients previously demonstrated to exist between these areas. Stable isotope gradients in the Bering Sea may have potential for predicting geographic distribution of King Eiders during wing molt.

keywords: *feather, marine, muscle, satellite transmitters, terrestrial*

INTRODUCTION

Declining population trends in King Eiders (*Somateria spectabilis*) and other sea duck species in recent decades have stimulated interest in the potentially vulnerable life stages during the non-breeding period of the annual cycle (U.S.F.W.S. 1999). These species, however, are most difficult to study at sea (Goudie and Ankney 1988) where they may spend more than 90% of their lives (Suydam 2000). A recent study of King Eiders migrating past Pt. Barrow, Alaska, estimated that by 1996 the North American population had declined to nearly half of the number that passed early in the 1970's (Suydam et al. 2000a).

King Eiders breeding in western North America migrate from their wintering areas in the Bering Sea during late April through May to nest on the North Slope of Alaska and the coastal plain of northern Canada in early June (Fig.1). Males generally spend less than two weeks on the breeding grounds, and females may remain an additional two months. Males begin to leave just before, or shortly after, the onset of incubation in June, and most have reached their molting areas by August (Cotter et al.

1997). Females begin leaving in mid-July through September and may be accompanied by hatch-year birds from late August until the end of migration in October (Suydam et al. 2000b). King Eiders wintering off the coast of Greenland are thought to undergo molt after migration from breeding areas, though it is unknown whether some females molt with fledging hatch-year birds (Frimer 1994). King Eiders simultaneously lose their flight feathers after arrival at molt sites and remain flightless for approximately three weeks until new feathers are synthesized (Bellrose 1976).

Satellite telemetry data and opportunistic observations from other studies in the Bering Sea have yielded important but limited information about the non-breeding period of King Eiders (Salomonsen 1968, Irving et al. 1970, Dickson et al. 2000, Suydam 2000). King Eiders breeding in the eastern Canadian Arctic were found to have wintered near the Chukotka Peninsula and eastern Bering Sea (Dickson et al. 1998, 1999, Phillips unpublished data). The high costs associated with satellite telemetry studies limit sample size and prevent population wide assessments. Due to logistical difficulties involved in conducting a study in the Bering Sea during winter, stable isotope analyses of tissues offer an alternative remote method to study the non-breeding period of King Eiders.

Stable isotopes of carbon and nitrogen from animal tissues are useful indicators of protein sources and trophic position in ecosystems. Fractionation distributes isotopes from the diet into newly formed tissue in such a way that isotopic composition of tissues closely reflects that of the diet (Deniro and Epstein 1978, 1981, Tieszen et al. 1983, Hobson and Clark 1992a, 1992b) and tissue isotope values increase with consecutive trophic level in a predictable manner (McConnaughey and McRoy 1979, Minagawa and

Wada 1984). Moreover, the use of $\delta^{13}C$ and $\delta^{15}N$ analyses in wildlife studies has been expanded to examination of migratory routes and origins (Kelly 2000, reviewed in Rubenstein and Hobson 2004). For example, because marine carbon sources are enriched in $\delta^{13}C$ relative to terrestrial sources (Tieszen and Boutton 1988), the $\delta^{13}C$ values of pectoral muscle from northern saw-whet owls (*Aegolius acadicus*) indicated that they derived protein for tissue synthesis from marine, terrestrial, or both ecosystems (Hobson and Sealy 1991). The $\delta^{13}C$ and $\delta^{15}N$ values of muscle from lesser snow geese (*Chen caerulescens caerulescens*) were traced back to dietary sources in the known wintering areas of these geese; thus, $\delta^{13}C$ and $\delta^{15}N$ values indicated that protein had been derived from three terrestrial biomes and reflected three subpopulations wintering in different locations (Alisauskas and Hobson 1993). Tissues that continue to grow turnover protein; thus, when there is a shift in diet, the stable isotopic composition of a tissue will be altered over time (Hobson 1999a). However, keratinous tissues, such as feathers, do not turnover protein and isotopic values are fixed after synthesis (Mizutani et al. 1992). The $\delta^{13}C$ and $\delta^{15}N$ values of head feathers from King Eiders collected during breeding in eastern Canada imply that this contiguous breeding population wintered in either the Atlantic or Pacific Ocean (Mehl et al. in press). The $\delta^{13}C$ values of natal down from dunlin (*Calidris alpina*) were very light relative to those of adult primaries (Klassaan et al. 2001) indicating that adults molt in different habitats than those used for brood rearing. Thus, while muscle tissue provides recent dietary information, feather sampling provides retrospective information on diet.

Invertebrates from the Bering, Chukchi, and Beaufort seas have $\delta^{13}C$ and $\delta^{15}N$ values that are isotopically heavier in the Bering Sea, become lighter along a gradient through the Chukchi Sea, and lighter still in the Beaufort Sea (Dunton et al. 1989, Schell et al. 1998, Hoekstra et al. 2002). In the Bering Sea, an east-west gradient in carbon isotopes has also been found in sediment samples (Naidu et al. 1993) that may be reflected throughout higher trophic levels, including King Eiders feathers produced in the Bering Sea.

The diet of King Eiders during molt consists primarily of marine invertebrates (Frimer 1997). Primary feathers that grow during flightless molt are synthesized from protein sources (i.e., marine invertebrates) reflecting the natural distributions of stable isotopes at a specific location. Therefore, isotopic information about diet during feather synthesis is stored until the next molt (Mizutani et al. 1992). Thus, $\delta^{13}C$ and $\delta^{15}N$ analyses of primary feathers from King Eiders could provide information about the diet and location of King Eiders during wing molt.

The objectives of this study were to (1) determine if $\delta^{13}C$ and $\delta^{15}N$ values of feathers from King Eiders could be explained by diet at wing molt location or bird gender, (2) compare $\delta^{13}C$ and $\delta^{15}N$ values in muscle and feather tissues, which might reflect shifts in diet from a marine-based diet during winter to a terrestrial based diet in summer and, (3) determine whether $\delta^{13}C$ values in feathers from transmittered eiders are correlated with known molt locations in the Bering Sea.

METHODS

We collected 14 King Eiders with a 12-gauge shotgun and wings from 258 migrating eiders harvested by subsistence hunters at Pt. Barrow, Alaska (71.3347° N, -156.7333° W) during spring (1 May- 1 June) and fall (11 July- 20 August) 2003. The sample collection consisted of 192 males and 93 females. We also included two females from 1996 and one from 1998. In addition, we collected primary feathers from nine male and three female King Eiders implanted with satellite transmitters at Kuparuk, Alaska (70.3172° N, -150.0008° W) prior to nesting in summer 2003. All birds were examined for plumage indicative of age status (adult vs. hatch-year/immature). Feathers were placed in paper envelopes and whole bodies were frozen until they were shipped to the University of Alaska, Fairbanks (UAF).

We processed tissue samples after the fall migration 2003. Feathers were cleaned and rinsed using ethanol to remove surface contaminants and air dried as described by Knoche et al. (in preparation). We used the ninth primary feather from each individual for consistency. We sampled breast muscle and feathers from different individuals in spring (n = 4 males, 4 females) and fall (n = 4 males, 9 females). Muscle tissue was rinsed with ethanol and freeze-dried for 24 hours. Freeze-dried samples were pulverized into a fine powder prior to isotopic analyses.

Geographic locations of wing molt for satellite transmittered King Eiders were determined using GIS analysis (ArcView GIS Version 3.3). We used the mean value of geographic coordinates for each individual's molt location. Molt location was defined as the period after molt migration when eiders did not change location for $\geq$ 3 weeks.

Variation in location during wing molt was small (<50 km^2) within the scale of the Bering Sea (2.3 x 10^6 km^2) (Hood and Kelley 1974). We standardized longitude because Universal Transverse Mercator (UTM) conversions are not available west of the central meridian 180°. Longitude was calculated using UTM Zone 3 False Easting as the origin because the most easterly molting King Eider molted in this zone. We added 500,000 m to each coordinate of longitude within a zone and added 500,000 m more for each consecutive zone (i.e. Zone 3 coordinates were not changed, Zone 2 + 500,000 m, Zone 1 + 1,000,000 m, Zone 60 + 1,500,000 m, etc.). The $\delta^{13}C$ and $\delta^{15}N$ of feathers from transmittered eiders were assumed to represent their wing molt locations in Fall 2002 because male King Eiders have been observed to show molt site fidelity (Salomonsen 1968, Frimer 1994). Coordinates from geographic molt locations in this study were from 2003 (Phillips unpublished data).

The $\delta^{13}C$ and $\delta^{15}N$ data were obtained using continuous flow stable isotope-ratio mass spectrometry. Samples were placed in tin boats (Elemental Microanalysis Limited, Devon, UK), combusted in a Carlo Erba NC2500 elemental analyzer (Carlo Erba, Milan, Italy) and released into a Finnigan Delta+XP (Thermo Electron Corporation, Waltham, MA). Stable isotope ratios were reported in δ notation, as parts per thousand (‰) relative to Pee Dee Belemnite (PDB) (carbon) and atmospheric air (nitrogen), using the following equation:

$$\delta X = ([R_{sample}/R_{standard}]\text{-}1 \text{ X } 1000,$$

where X is ^{15}N or ^{13}C and R is the ratio of ^{15}N:^{14}N or ^{13}C:^{12}C. Instrument precision was determined by conducting multiple analyses of a homogenous calibrated peptone standard (n = 29) ($\delta^{13}C$ = 7.0 ‰, C = 44.3 %, $\delta^{15}N$ = 15.8 ‰, N =15.8 %) and was <±0.4 ‰ for $\delta^{13}C$, <±0.3 ‰ for $\delta^{15}N$.

We used the Analysis of Variance using SAS Proc GLM (SAS Institute 1996) to compare differences in $\delta^{13}C$ and $\delta^{15}N$ values due to gender or location of molt. We compared $\delta^{13}C$ and $\delta^{15}N$ values from feathers and tissues using a three-way ANOVA including tissue type, season (Fall, Spring), and gender to examine dietary shifts. We used SAS Proc Reg to determine the relationship between transmittered male wing molt locations and primary feather stable isotope values. Means were reported with ±1 S.D.

RESULTS

Ninth primary feathers from all King Eiders sampled demonstrated a wide range of $\delta^{13}C$ values (mean = -17.94 ± 1.68 ‰, range = -14.67 to -25.55 ‰) and $\delta^{15}N$ values (mean = 14.91 ± 1.92 ‰, range = 5.52 to 20.27 ‰) (Appendix). Feather $\delta^{13}C$ values did not differ within or between genders (Fig. 2). Feather $\delta^{15}N$ values did not vary among males. Nine females had lighter $\delta^{15}N$ of feathers values relative to the majority of females (n = 99, $P < 0.001$) and all males (n =188, $P < 0.001$, Fig. 2) even though the groups did not differ in $\delta^{13}C$ values. These same nine females also had the lightest $\delta^{13}C$ values (mean = -24.41 ± 1.37) (Fig. 2). Six of this group were collected in 2003, two in 1996, and one in 1998. All plumages were representative of adult females in this group

and the female collected in 1998 had a brood patch, indicating breeding during the year of collection.

We detected no within season differences in $\delta^{13}C$ and $\delta^{15}N$ values of feathers (Fig. 3). Muscle tissue was isotopically heavier in spring than it was in the fall ($n = 13$, $\delta^{15}N$ $P < 0.001$ and $\delta^{13}C$ $P < 0.03$, Fig. 3). In spring, the $\delta^{13}C$ and $\delta^{15}N$ compositions of muscle and feather did not differ between tissues. There was a significant tissue by season interaction where muscle $\delta^{13}C$ and $\delta^{15}N$ ($n = 17$, $P < 0.001$, Fig. 3) were isotopically lighter in fall than spring. Gender was not significant in the tissue comparisons.

The distribution of molting nine male King Eiders equipped with satellite transmitters ranged from 58.5134° N, -158.3051° W (NAD-27) at Bristol Bay, Alaska to 60.8514° N, 171.9665° W at the west coast of Siberia. The $\delta^{13}C$ values of feathers from those males were positively correlated with longitude of wing molt location in the Bering Sea ($n = 9$, $R^2 = 0.802$, Fig. 4), that is, feather $\delta^{13}C$ values were increasingly enriched along an east-west gradient in the Bering Sea. There was no relationship between $\delta^{15}N$ values of these males or between $\delta^{13}C$ and $\delta^{15}N$ values of feathers from female transmittered eiders and known molt locations.

DISCUSSION

Differences in feather $\delta^{15}N$ values among female King Eiders likely resulted from feeding in different ecosystems (i.e. terrestrial vs. marine) and did not result from gender differences of isotopic fractionation because many males and females overlapped. We found no gender differences in $\delta^{13}C$ and $\delta^{15}N$ values of feather and muscle tissues in this

study or in feathers from captive King Eiders reared on a uniform diet (Knoche et al. in preparation), corroborating this conclusion. The majority of female King Eiders in this study also demonstrated heavier, 'marine', $\delta^{13}C$ and $\delta^{15}N$ values that were similar to those of males. If genders exhibit similar isotopic fractionation, then any differences would suggest a difference in locations of wing molt. We suggest that the nine isotopically light females molted their primary feathers at terrestrial breeding areas rather than marine locations. It is not uncommon for birds of the same species to have distinct $\delta^{15}N$ values in feathers if the isotopic signatures of the biome where feathers were molted are different (Hobson 1999b). Endogenous contributions to eggs from large bodied migratory birds typically had higher $\delta^{15}N$ values relative to exogenous contributions because the marine diet sources tend to be enriched in ^{15}N relative to terrestrial sources (Hobson et al. 1997). Therefore, females that derive protein from freshwater sources during molt would have lower feather $\delta^{15}N$ values relative to those of females that synthesized feathers from marine protein sources. Though $\delta^{13}C$ values did not vary significantly within or between genders, those of female King Eiders with lower $\delta^{15}N$ values also had lower $\delta^{13}C$ values that were indicative of terrestrial protein sources (Tieszen and Boutton 1988, Mizutani et al. 1990, Bearhop et al. 1999).

Of the nine female King Eiders with terrestrial $\delta^{13}C$ and $\delta^{15}N$ values, six were sampled during the spring and fall migration of 2003, representing 6% of the total females collected that year. The same proportion of females passing by Pt. Barrow up to the last collection date in 2003 (Suydam unpublished data) was approximately 9600 females that could have potentially molted in terrestrial areas on the Arctic coast prior to

fall migration. We would expect to detect an even greater proportion of females with terrestrial stable isotope values if breeding females were included in our sample. We were not able to sample this portion of the population in 2003 because weather reduced hunter success in September (personal observation). We considered the possibility that females with 'terrestrial' isotope signatures could be hatch-year birds that would be expected to have stable isotope values reflecting molt sites in the breeding areas, and we provide evidence that they were adults: (1) females were all shot by subsistence hunters prior to 20 August before hatch-year King Eiders migrate past Pt. Barrow (usually late August or early September) (Suydam et al. 2000b), (2) one of the females with terrestrial $\delta^{13}C$ and $\delta^{15}N$ values was collected in late September 1998 had a brood patch, and (3) there was no plumage indication of juveniles.

Differences in muscle $\delta^{13}C$ and $\delta^{15}N$ values between seasons in the same individuals reflected dietary shifts that are inherent when birds migrate between regions with distinct isotopic abundance (Hobson et al. 2000, Klassaan et al. 2001, Morrison and Hobson 2004). Muscle $\delta^{13}C$ and $\delta^{15}N$ values in the fall then reflect protein turnover that occurred after spring migration and prior to fall migration (i.e., during breeding). Muscle tissue from male and non-breeding female eiders collected in the fall could be influenced by the geographic gradients in stable isotope distribution in the Bering, Chukchi, and Beaufort Seas (Dunton 1989, Schell et al. 1998) and potentially from terrestrial sources during breeding attempts. Depending on the origin of migration, Pt. Barrow, Alaska, is generally less than half the of the total migration distance for eiders in the spring and can be reached in a few days (Suydam et al. 2000b). Eiders departing

wintering areas should then have a muscle and tissue stable isotopic composition reflecting the isotopically heavier marine environment. Given that the rate of stable isotope turnover is slow for muscle (several weeks) (Hobson 1999a), muscle and feather tissues collected from King Eiders in the spring would not be necessarily be expected to have different isotope values because the passage of time is abbreviated between the departure from the origin of migration and our sampling at Pt. Barrow. Comparisons of tissue type also provided evidence for the dietary shift because feather composition is fixed at the time of synthesis and, thus, feathers will reflected marine derived protein sources unless molt occurred in terrestrial areas.

The $\delta^{13}C$ values of feathers synthesized in Fall 2002 from male King Eiders implanted with satellite transmitters were correlated with longitude of their wing molt locations in the Bering Sea in winter 2003. The $\delta^{13}C$ values of Bering Sea sediments show a gradient from the Seward Peninsula (lighter values) to the south of the Chukotka Peninsula, Russia (heavier values) (Naidu et al. 1993). Assuming that particulate organic carbon found in sediments is the basic carbon source in marine systems (Fry and Scherr 1984) it is not surprising to encounter the same trend in marine fauna (see Schell et al. 1998), including eiders. One transmittered King Eider molted several hundred kilometers north of the Kamchatka Peninsula, Russia, in an area not previously sampled for stable isotope studies. This particular male had very high $\delta^{13}C$ values and low $\delta^{15}N$ in feathers, indicating a protein source that was proportionally enriched in ^{13}C relative to ^{15}N. One possible explaination is that the male was feeding in a lower trophic level in the vicinity of benthic macrophytes which produce higher $\delta^{13}C$ values relative to phytoplankton

(France 1995). We believe that feather stable isotope values from female King Eiders did not reflect known isotopic gradients for several reasons. Recently, stable isotope values of head feathers from King Eiders indicated that some females alternated wintering areas between the Atlantic and Pacific Oceans (Mehl et al. 2004). Heterogeneity of winter site fidelity would also potentially affect molt site fidelity, particularly with geographic variations of the magnitude found by Mehl et al. (2004). Additionally, the small sample size of females in our study could not have provided a basis for any analysis. Though feathers from male King Eiders demonstrated that $\delta^{13}C$ values were correlated with the longitude of their wing molt location, we cannot assess this relationship for females without a larger number of samples.

To summarize, the $\delta^{13}C$ and $\delta^{15}N$ values of feather and/or muscle tissues provided new information regarding wing molt strategies, seasonal dietary shifts, and the geographic distribution of King Eiders. First, we estimated that ~6% of females may molt prior to leaving breeding areas, which introduces an additional species to consider when managing Arctic coastal areas for resource development. Our estimate does not include breeding females and their offspring that migrate past Barrow in September during peak migration for females (Suydam et al. 2000b), but suggests we underestimated the number of King Eiders using breeding areas to molt because we could not sample late-migrating females or hatch-year birds. Similarly, females that molted in the Beaufort Sea would not be easily discernible from those molting in the Bering Sea because of the large variation in marine stable isotope feather values. We also concluded that comparisons of $\delta^{13}C$ and $\delta^{15}N$ values of muscle between Spring and Fall migration

demonstrated that after northward migration to breeding area, the diets of King Eiders were isotopically depleted relative to their winter diet. Lighter muscle values reflected either a switch to terrestrial/freshwater carbon and nitrogen sources if a breeding attempt was made or simply switching between the isotopically distinct marine food webs of the Bering and Beaufort Seas during staging. Finally, correspondence of the $\delta^{13}C$ values of primary feathers in transmittered males and the known geographic wing molt location provides a longitudinal component for determination of wing molt or wintering locations. Coupled with another stable isotope or latitudinal component, approximate range maps for these events could be produced. The findings of this study further promote the use of feathers in examining movements and trophic ecology of birds. We have revealed a better understanding of the patterns of wing molt in King Eiders in that some individuals may be using inshore and terrestrial areas in the Beaufort Sea, an unexpected finding.

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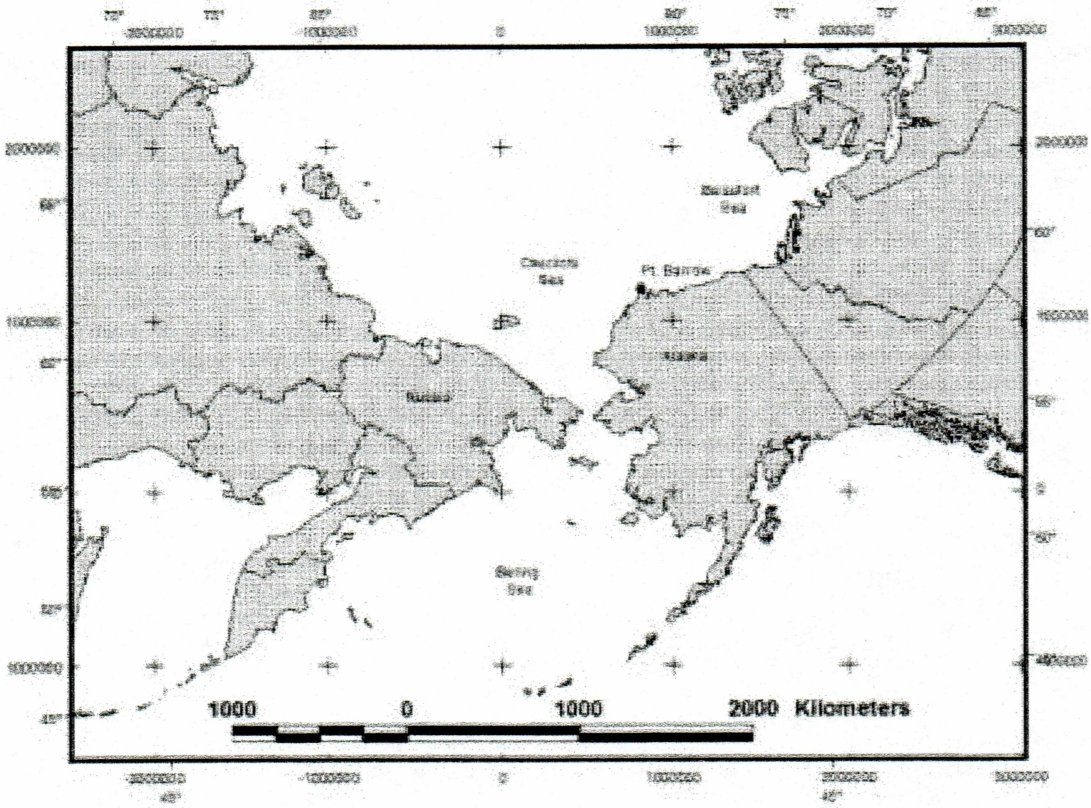

Figure 1. Range map of the population of King Eiders that Migrate past Pt. Barrow, AK.

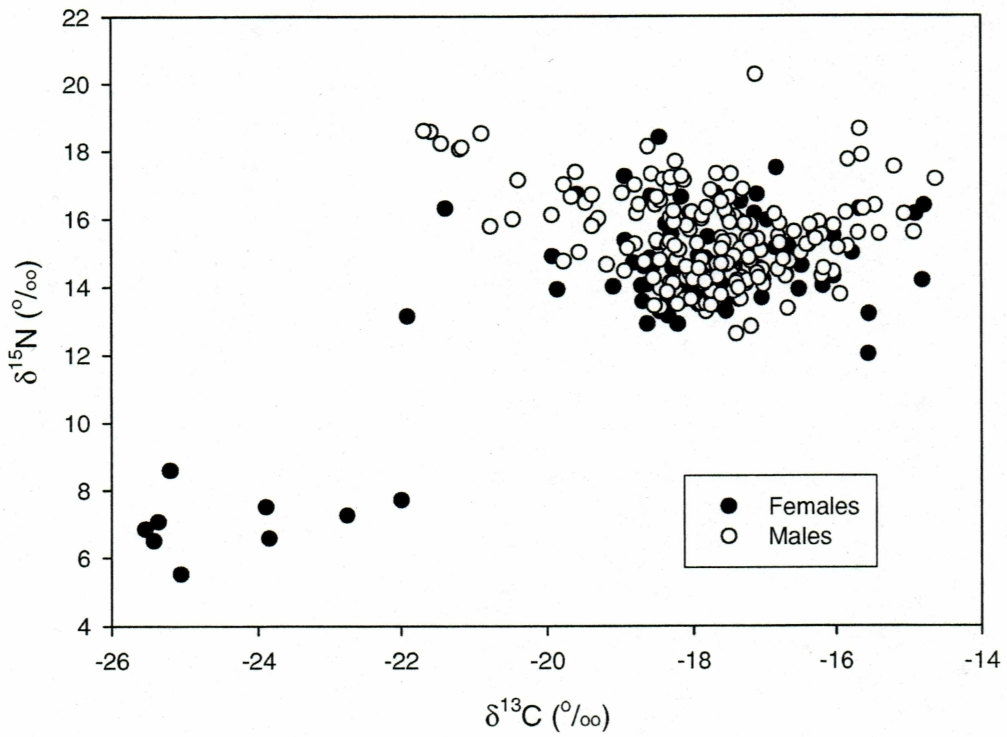

FIGURE 2. $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers from King Eiders. Feather samples (n = 287) were collected during spring and fall migration at Pt. Barrow, Alaska, and transmittered near their arrival on breeding areas at Kuparuk, Alaska.

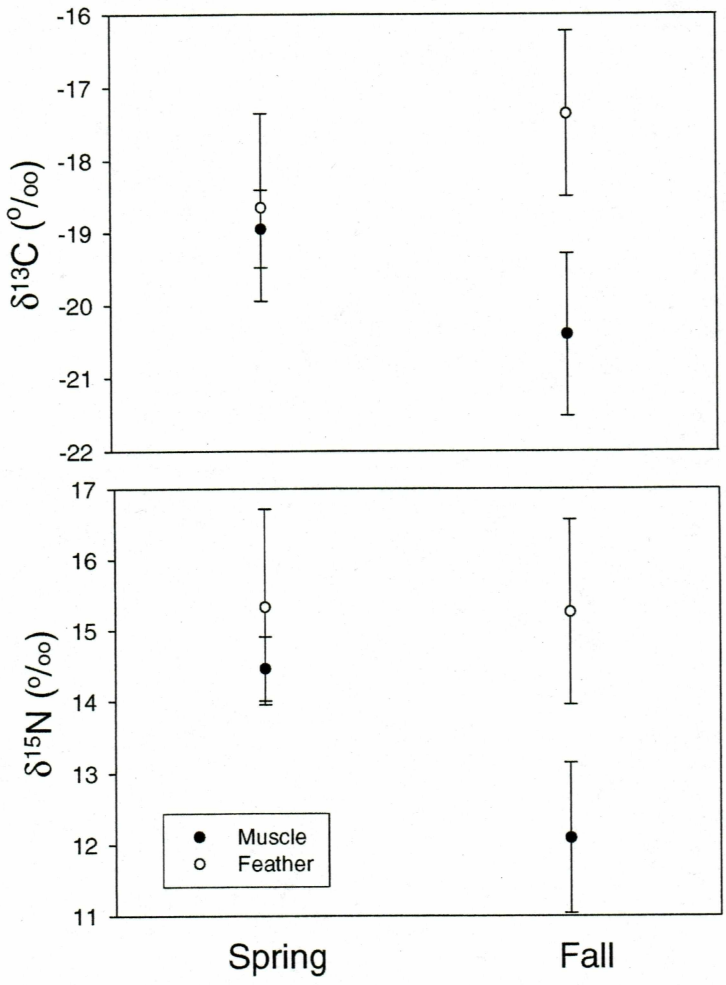

FIGURE 3. Seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ values of feather and muscle. Mean tissue values are presented for $\delta^{13}C$ and $\delta^{15}N$ from King Eider tissue samples collected from different individuals in spring (n = 4 males, 4 females) and fall (n = 4 males, 9 females) 2003. Means are reported with ± 1 S.D.

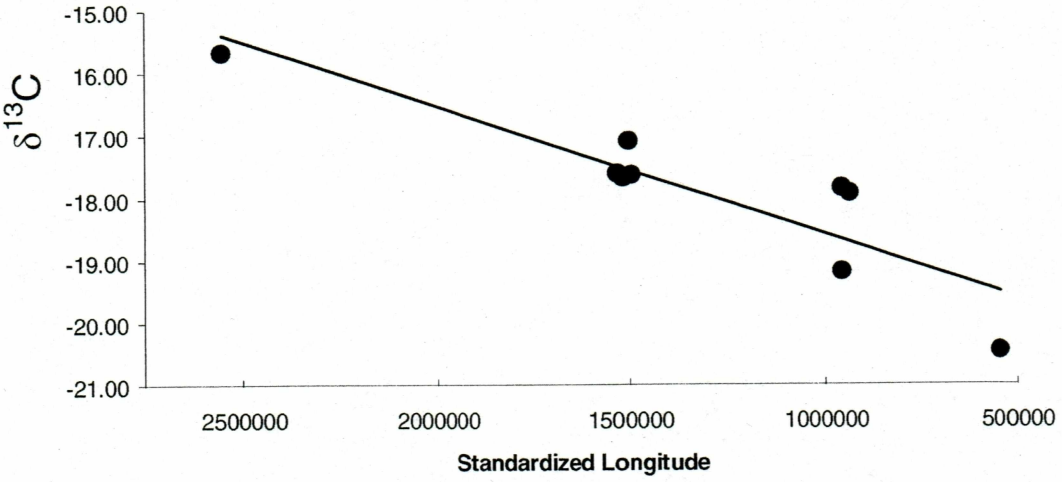

FIGURE 4. The $\delta^{13}C$ gradient of male eider molt locations in the Bering Sea. Distribution of $\delta^{13}C$ values of primary feathers from male King Eiders collected in 2003 plotted against standardized longitude of 2002 wing molt for those males.

GENERAL CONCLUSIONS

We have improved our understanding of the wing molting ecology of King Eiders with the use of stable isotope analyses of their primary feathers. The captive eider experiment resulted in methods that can be used across avian taxa, as well as insights that can be applied to data from wild eiders. The application of stable isotope analyses also enabled us to obtain data regarding remote locations of molt from wild eiders that would not otherwise be possible.

Comparisons of cleaning methods to remove surface contaminants from feathers implemented in this study demonstrated that the methods applied did not influence $\delta^{13}C$ and $\delta^{15}N$ values significantly. As a result, our suggestion to use ethanol can be applied to clean feathers of all types and across avian taxa. Another noteworthy result from the cleaning study was that isotopic values of the feather did not vary as a function of distance from the base of the feather. We concluded that diets did not change during feathers synthesis. Though this is not surprising in captive birds, its implications are important for the analyses of primary feathers in wild eiders and the location of their wing molt.

Results stemming from the examination of $\delta^{13}C$ and $\delta^{15}N$ values of fractionation from diet corresponded with previous studies. However, the effects of age on fractionation from diet to tissue have only been alluded to in the literature (Hobson and Clark 1992b, Graves et al. 2002). The AHY birds had $\delta^{13}C$ and $\delta^{15}N$ fractionation values that did not differ by gender, and were within predicted ranges from previous studies (Hobson and Clark 1992a, Mizutani et al. 1992, reviewed in Kelly 2000, Pearson et al.

2003). However, the close correspondence between the $\delta^{15}N$ values from feathers and diet of HY eiders suggested that growth may effect isotopic fractionation. This contribution to the stable isotope literature could instigate further investigation of effects of elevated metabolic rates and/or growth on isotopic fractionation.

The $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers from wild King Eiders generated new information regarding molt strategies. Generally, there appeared to be no gender differences or geographic separation during molt at sea. Stable isotope values also demonstrated that some eiders of both genders molted in mixed aquatic systems. Additionally, the $\delta^{13}C$ and $\delta^{15}N$ values revealed that a small proportion of female King Eiders underwent wing molt in the terrestrial habitats, which has not been noted for this species (Frimer 1994, Suydam 2000). Female eiders with broods have been observed molting at sea along the Arctic coast (Parmelee et al. 1967). Mixed-aquatic $\delta^{13}C$ and $\delta^{15}N$ values of primary feathers cannot discriminate between molts in the Beaufort Sea prior to migration or in the Bering Sea after migration.

Finally, correspondence between $\delta^{13}C$ values of feathers from satellite transmittered male King Eiders and their known molt locations provided information about the geographic distribution of King Eiders during molt. Location may be assessed using stable isotope analyses only if other variables, such as molting sequence, are fixed to occur during a specific time period during the annual cycle. Though timing primary feather molt sequence in eiders is fixed to occur in the Fall, males undergo molt at the same location on an annual basis and, therefore, could potentially be used to determine location. Previously, it was suggested that regional isotope maps could be generated

using stable isotopes as geographical (x,y) coordinates (Wassenaar and Hobson 2001). Such maps do not produce data that is as precise as satellite telemetry, but the low cost and simplicity of feather sampling yields larger sample sizes and is more applicable to population-scale assessment. Furthermore, the gradient found for $\delta^{13}C$ values of feathers mirrored the same gradients as sediments in the Bering Sea (Naidu et al. 1993). The fact that this large geographic gradient is reflected in higher trophic levels and persistent over time is novel, and may provide support for the use of stable isotope analyses for other migratory species inhabiting these same regions.

I recommend that future studies using stable isotope analyses to examine King Eider biology should include: (1) examination of amino acid transfer from diet to tissues during growth that might help explain differences in fractionation values between AHY and HY eiders, and (2) focus on delineating the proportion of eiders molting in mixed aquatic systems in the Bering and Beaufort Seas. To examine amino acid transfer from diet to tissue in AHY and HY eiders, I suggest rearing both age groups on two isotopically and nutritionally uniform diets (i.e. diet A: light $\delta^{15}N$ and low dietary N, diet B: high $\delta^{15}N$ and high dietary N). Both diet and feathers should be analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values. The addition of $\delta^{34}S$ from diet and feather would help to understand the transfer of essential sulfur containing amino acids from diet to feather. The study could also be improved with systematic blood sampling before, during, and after molt. Examination of $\delta^{13}C$, $\delta^{15}N$ values of amino acids in blood serum would produce current (i.e. that day) information regarding isotopic fractionation from diets to tissues in AHY and HY eiders while the signatures of red blood cells, which turnover

protein at a slower rate, would reflect any variation in amino acid transfer during molt. Time series data would potentially increase precision of determining age-dependent difference due to growth. Precision of using $\delta^{13}C$ and $\delta^{15}N$ values of feathers to determine molt distribution would be improved by obtaining diet samples from known molt locations of wild birds. Previous studies have used dietary samples from wild birds to determine the proportion of populations feeding in terrestrial, marine, or mixed aquatic systems (Hobson 1990, Bearhop et al. 1999). Collection of dietary samples from the isotopically distinct Bering and Beaufort Seas has potential to delineate groups of eiders that molted at sea in the Bering or Beaufort Sea.

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APPENDIX A. The $\delta^{15}N$ and $\delta^{13}C$ values of feathers from individual King Eiders. Samples were collected with A shotgun (Collection), from subsistence hunters (Subsistence), or from eiders implanted with satellite transmitters (satellite), during spring and fall at Pt. Barrow and Kuparuk, AK in 2003.

ID	Gender	Season	Type	$\delta^{15}N$	$\delta^{13}C$
1	F	Spring	Collection	15.28	-18.48
2	F	Spring	Collection	14.25	-17.73
3	F	Spring	Collection	15.36	-18.93
4	F	Fall	Collection	14.91	-17.93
5	F	Fall	Collection	15.50	-16.03
6	F	Fall	Collection	14.18	-14.80
7	F	Fall	Collection	13.64	-17.52
8	F	Spring	Collection	16.30	-21.38
9	F	Spring	Collection	12.91	-18.63
10	F	Spring	Collection	16.14	-17.15
11	F	Spring	Collection	16.16	-18.04
12	F	Spring	Collection	15.14	-16.85
13	F	Spring	Collection	13.92	-16.51
14	F	Spring	Collection	13.27	-18.45
15	F	Spring	Subsistence	15.83	-18.37
16	F	Spring	Subsistence	16.28	-15.67
17	F	Spring	Subsistence	13.77	-18.06
18	F	Spring	Subsistence	13.67	-17.04
19	F	Spring	Subsistence	14.00	-19.10
20	F	Spring	Subsistence	16.03	-18.30
21	F	Spring	Subsistence	14.31	-17.79
22	F	Spring	Subsistence	14.84	-18.59
23	F	Spring	Subsistence	14.53	-18.00
24	F	Spring	Subsistence	14.71	-18.82
25	F	Fall	Subsistence	7.07	-25.37
26	F	Fall	Subsistence	7.71	-21.99
27	F	Fall	Subsistence	6.85	-25.55
28	F	Fall	Subsistence	6.51	-25.43
29	F	Fall	Subsistence	8.59	-25.21
30	F	Fall	Subsistence	7.51	-23.90
31	F	Fall	Subsistence	7.26	-22.76

32	F	Fall	Subsistence	14.16	-17.65
33	F	Fall	Subsistence	14.22	-17.02
34	F	Fall	Subsistence	15.46	-17.80
35	F	Fall	Subsistence	16.37	-14.78
36	F	Fall	Subsistence	13.68	-18.01
37	F	Fall	Subsistence	12.02	-15.54
38	F	Fall	Subsistence	16.66	-18.57
39	F	Fall	Subsistence	14.54	-17.34
40	F	Fall	Subsistence	13.84	-17.90
41	F	Fall	Subsistence	13.88	-17.56
42	F	Fall	Subsistence	13.46	-17.64
43	F	Fall	Subsistence	15.08	-17.05
44	F	Fall	Subsistence	13.69	-18.34
45	F	Fall	Subsistence	14.40	-18.01
46	F	Fall	Subsistence	13.15	-18.34
47	F	Fall	Subsistence	15.18	-16.66
48	F	Fall	Subsistence	18.40	-18.46
49	F	Fall	Subsistence	5.52	-25.07
50	F	Fall	Subsistence	13.85	-18.03
51	F	Fall	Subsistence	16.13	-14.89
52	F	Fall	Subsistence	13.28	-17.54
53	F	Fall	Subsistence	13.47	-17.93
54	F	Fall	Subsistence	14.55	-18.28
55	F	Fall	Subsistence	15.22	-16.38
56	F	Fall	Subsistence	13.19	-15.53
57	F	Fall	Subsistence	12.90	-18.21
58	F	Fall	Subsistence	16.63	-18.16
59	F	Fall	Subsistence	15.53	-18.30
60	F	Fall	Subsistence	14.71	-17.78
61	F	Fall	Subsistence	14.93	-17.21
62	F	Fall	Subsistence	14.36	-17.83
63	F	Fall	Subsistence	15.47	-16.30
64	F	Fall	Subsistence	15.68	-16.29
65	F	Fall	Subsistence	14.28	-17.81
66	F	Fall	Subsistence	13.93	-17.66
67	F	Fall	Subsistence	14.90	-19.92
68	F	Fall	Subsistence	14.56	-18.56
69	F	Fall	Subsistence	16.71	-17.11
70	F	Fall	Subsistence	14.54	-17.97
71	F	Fall	Subsistence	14.99	-15.77

72	F	Fall	Subsistence	14.43	-17.16
73	F	Fall	Subsistence	17.49	-16.83
74	F	Fall	Subsistence	14.88	-17.24
75	F	Fall	Subsistence	16.50	-17.33
76	F	Fall	Subsistence	13.28	-18.38
77	F	Fall	Subsistence	6.58	-23.85
78	F	Fall	Subsistence	16.72	-19.59
79	F	Fall	Subsistence	14.16	-18.10
80	F	Fall	Subsistence	14.32	-16.69
81	F	Fall	Subsistence	14.06	-18.63
82	F	Fall	Subsistence	15.95	-16.97
83	F	Fall	Subsistence	14.83	-17.84
84	F	Fall	Subsistence	13.57	-18.69
85	F	Fall	Subsistence	14.03	-18.71
86	F	Fall	Subsistence	14.02	-16.18
87	F	Fall	Subsistence	17.25	-18.94
88	F	Fall	Subsistence	14.36	-17.97
89	F	Fall	Subsistence	16.76	-17.69
90	F	Fall	Subsistence	13.93	-19.85
91	F	Fall	Subsistence	14.30	-16.03
92	F	Fall	Subsistence	13.14	-21.91
93	F	Fall	Subsistence	14.61	-16.48
94	M	Fall	Subsistence	16.62	-18.51
95	M	Fall	Subsistence	16.37	-15.45
96	M	Spring	Collection	14.26	-18.09
97	M	Spring	Collection	15.85	-19.36
98	M	Spring	Collection	17.31	-18.57
99	M	Spring	Subsistence	15.35	-18.50
100	M	Spring	Subsistence	13.98	-18.38
101	M	Spring	Subsistence	16.75	-18.97
102	M	Spring	Subsistence	15.25	-18.80
103	M	Spring	Subsistence	15.16	-18.25
104	M	Spring	Subsistence	15.42	-17.61
105	M	Spring	Subsistence	15.65	-16.19
106	M	Spring	Subsistence	17.69	-18.24
107	M	Spring	Subsistence	13.62	-18.12
108	M	Spring	Subsistence	14.63	-17.73
109	M	Spring	Subsistence	14.25	-17.44
110	M	Spring	Subsistence	15.78	-17.20
111	M	Spring	Subsistence	14.61	-17.56

112	M	Spring	Subsistence	13.77	-15.94
113	M	Spring	Subsistence	14.58	-17.23
114	M	Fall	Subsistence	20.27	-17.13
115	M	Fall	Subsistence	14.78	-17.88
116	M	Fall	Subsistence	15.98	-17.88
117	M	Fall	Subsistence	17.13	-20.38
118	M	Fall	Subsistence	15.02	-19.56
119	M	Fall	Subsistence	13.63	-17.37
120	M	Fall	Subsistence	18.53	-20.88
121	M	Fall	Subsistence	14.25	-17.32
122	M	Fall	Subsistence	14.08	-17.27
123	M	Fall	Subsistence	13.76	-17.64
124	M	Fall	Subsistence	14.56	-17.49
125	M	Fall	Subsistence	16.78	-17.33
126	M	Fall	Subsistence	16.12	-15.04
127	M	Fall	Subsistence	16.19	-18.02
128	M	Fall	Subsistence	13.27	-17.82
129	M	Fall	Subsistence	15.46	-16.80
130	M	Fall	Subsistence	13.39	-18.45
131	M	Fall	Subsistence	13.50	-17.90
132	M	Fall	Subsistence	14.38	-18.04
133	M	Fall	Subsistence	14.05	-17.02
134	M	Fall	Subsistence	14.31	-16.70
135	M	Fall	Subsistence	17.16	-14.62
136	M	Fall	Subsistence	16.65	-19.66
137	M	Fall	Subsistence	13.63	-17.34
138	M	Fall	Subsistence	16.00	-19.30
139	M	Fall	Subsistence	17.52	-15.18
140	M	Fall	Subsistence	13.36	-16.67
141	M	Fall	Subsistence	16.17	-15.85
142	M	Fall	Subsistence	13.50	-17.82
143	M	Fall	Subsistence	14.99	-16.49
144	M	Fall	Subsistence	16.03	-18.31
145	M	Fall	Subsistence	16.84	-17.76
146	M	Fall	Subsistence	13.49	-18.22
147	M	Fall	Subsistence	16.11	-19.93
148	M	Fall	Subsistence	14.36	-17.96
149	M	Fall	Subsistence	14.68	-17.59
150	M	Fall	Subsistence	13.63	-17.74
151	M	Fall	Subsistence	15.83	-17.20

152	M	Fall	Subsistence	15.60	-16.58
153	M	Fall	Subsistence	12.83	-17.20
154	M	Fall	Subsistence	17.00	-19.76
155	M	Fall	Subsistence	16.41	-18.52
156	M	Fall	Subsistence	15.05	-17.06
157	M	Fall	Subsistence	13.95	-18.44
158	M	Fall	Subsistence	15.78	-19.38
159	M	Fall	Subsistence	16.28	-15.61
160	M	Fall	Subsistence	13.66	-17.65
161	M	Fall	Subsistence	15.78	-20.76
162	M	Fall	Subsistence	15.13	-18.90
163	M	Fall	Subsistence	14.64	-17.44
164	M	Fall	Subsistence	15.54	-15.39
165	M	Fall	Subsistence	17.73	-15.82
166	M	Fall	Subsistence	17.86	-15.63
167	M	Fall	Subsistence	15.66	-18.05
168	M	Fall	Subsistence	13.91	-17.41
169	M	Fall	Subsistence	15.17	-15.84
170	M	Fall	Subsistence	15.94	-17.37
171	M	Fall	Subsistence	14.36	-17.47
172	M	Fall	Subsistence	15.23	-18.35
173	M	Fall	Subsistence	15.78	-18.09
174	M	Fall	Subsistence	14.17	-17.39
175	M	Fall	Subsistence	15.32	-18.32
176	M	Fall	Subsistence	14.46	-18.94
177	M	Fall	Subsistence	16.85	-17.31
178	M	Fall	Subsistence	14.81	-17.38
179	M	Fall	Subsistence	15.88	-18.26
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181	M	Fall	Subsistence	14.88	-17.39
182	M	Fall	Subsistence	14.54	-17.07
183	M	Fall	Subsistence	17.37	-19.60
184	M	Fall	Subsistence	16.61	-17.50
185	M	Fall	Subsistence	16.47	-19.47
186	M	Fall	Subsistence	14.27	-17.75
187	M	Fall	Subsistence	14.33	-17.67
188	M	Fall	Subsistence	14.93	-18.32
189	M	Fall	Subsistence	15.58	-14.92
190	M	Fall	Subsistence	16.16	-18.77
191	M	Fall	Subsistence	17.13	-18.13

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195	M	Fall	Subsistence	16.05	-17.43
196	M	Fall	Subsistence	15.89	-16.23
197	M	Fall	Subsistence	14.17	-17.25
198	M	Fall	Subsistence	14.31	-16.19
199	M	Fall	Subsistence	14.53	-16.17
200	M	Fall	Subsistence	14.78	-18.23
201	M	Fall	Subsistence	14.50	-16.81
202	M	Fall	Subsistence	15.33	-17.48
203	M	Fall	Subsistence	14.73	-16.65
204	M	Fall	Subsistence	16.05	-17.51
205	M	Fall	Subsistence	13.86	-17.44
206	M	Fall	Subsistence	17.32	-17.67
207	M	Fall	Subsistence	14.77	-18.45
208	M	Fall	Subsistence	15.13	-15.97
209	M	Fall	Subsistence	18.22	-21.44
210	M	Fall	Subsistence	16.99	-18.80
211	M	Fall	Subsistence	14.09	-17.86
212	M	Fall	Subsistence	16.07	-17.88
213	M	Fall	Subsistence	15.22	-16.41
214	M	Fall	Subsistence	14.10	-17.40
215	M	Fall	Subsistence	14.58	-18.10
216	M	Fall	Subsistence	17.15	-18.41
217	M	Fall	Subsistence	15.26	-17.94
218	M	Fall	Subsistence	18.58	-21.58
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222	M	Fall	Subsistence	13.44	-18.53
223	M	Fall	Subsistence	14.09	-18.27
224	M	Fall	Subsistence	18.12	-18.61
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226	M	Fall	Subsistence	16.89	-18.31
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230	M	Fall	Subsistence	14.32	-17.06
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233	M	Fall	Subsistence	14.21	-17.78
234	M	Fall	Subsistence	14.65	-17.54
235	M	Fall	Subsistence	14.49	-17.03
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242	M	Fall	Subsistence	13.84	-18.35
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244	M	Fall	Subsistence	18.61	-21.68
245	M	Fall	Subsistence	15.79	-16.03
246	M	Fall	Subsistence	15.40	-17.10
247	M	Fall	Subsistence	15.27	-16.78
248	M	Fall	Subsistence	17.31	-17.47
249	M	Fall	Subsistence	14.67	-17.52
250	M	Fall	Subsistence	16.62	-18.49
251	M	Fall	Subsistence	13.45	-17.75
252	M	Fall	Subsistence	16.22	-18.27
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254	M	Fall	Subsistence	15.85	-16.79
255	M	Fall	Subsistence	18.63	-15.66
256	M	Fall	Subsistence	14.38	-17.11
257	M	Fall	Subsistence	15.33	-17.21
258	M	Fall	Subsistence	16.32	-17.81
259	M	Fall	Subsistence	13.95	-17.37
260	M	Fall	Subsistence	15.08	-18.19
261	M	Fall	Subsistence	14.75	-19.77
262	M	Fall	Subsistence	15.19	-18.25
263	M	Fall	Subsistence	13.63	-18.03
264	M	Fall	Subsistence	12.62	-17.40
265	M	Fall	Subsistence	16.41	-18.74
266	M	Fall	Subsistence	16.49	-17.61
267	M	Fall	Subsistence	14.66	-17.67
268	M	Fall	Subsistence	14.25	-18.54
269	M	Fall	Subsistence	16.70	-19.38
270	M	Fall	Subsistence	14.20	-17.81
271	M	Fall	Subsistence	14.85	-17.22

272	M	Fall	Subsistence	17.24	-18.16
273	M	Fall	Subsistence	16.13	-16.86
274	M	Fall	Subsistence	15.88	-17.49
275	M	Fall	Subsistence	15.84	-17.28
276	F	Pre-Breeding	Satellite	14.60	-18.67
277	F	Pre-Breeding	Satellite	13.83	-17.89
278	F	Pre-Breeding	Satellite	14.57	-18.14
279	M	Pre-Breeding	Satellite	15.57	-15.68
280	M	Pre-Breeding	Satellite	13.75	-17.61
281	M	Pre-Breeding	Satellite	14.28	-17.67
282	M	Pre-Breeding	Satellite	14.25	-17.09
283	M	Pre-Breeding	Satellite	14.66	-17.61
284	M	Pre-Breeding	Satellite	15.98	-20.45
285	M	Pre-Breeding	Satellite	14.53	-17.92
286	M	Pre-Breeding	Satellite	14.65	-19.18
287	M	Pre-Breeding	Satellite	14.15	-17.84